APPENDIX A

Water Quality Procedures

- Sequential Comparison Index
- Beck's Biotic Index
- Floating Body Technique

Sequential Comparison Index

The Sequential Comparison Index (SCI) is a simple stream quality method, based upon distinguishing organisms by color, size, and shape, and requires no taxonomic expertise (ref. 8-4). The only needs are to be able to distinguish the number of different types (taxa) of organisms and the number of "runs" in samples containing less than 250 organisms. A diversity index (DI) is obtained by dividing the number of runs by the number of specimens. This index is multiplied by the number of taxa to give the final DI. DI values of 12 or above are indicative of healthy streams with high diversity and a balanced density. Polluted streams typically have DI values of 8 or less.

Sample analysis. There are many methods of biological specimen analysis. Diversity indices are useful because they condense considerable data into a single numerical value. The SCI is a simple diversity method which can be used by a non-biologist. The following is a brief summary of the SCI evaluation. For detailed information see Cairns' article on simple biological assessment (ref. 5-5).

Bottom sample collection and preservation. Bottom samples from a watercourse or water body should be collected with an appropriate sampler. If a bottom sampler is not available, trowels or shovels can be used to collect the sample. Place the material collected into a tub or bucket. Dilute the material with water and swirl. Pour it through a U.S. Standard #30 sieve or a 30-mesh screen. Remove rocks, sticks, and other artifacts after carefully checking for clinging organisms. Wash the screened material into a container and preserve it in 10 percent formalin or 70 percent ethanol (ethyl alcohol). Organisms may be sorted from the sample detritus in the field with forceps or at the laboratory. It is often desirable, prior to preserving the sample, to place rocks, sticks, and other objects in a white pan partially filled with water. Many of the animals will float free from the objects or can be removed with forceps. All samples should be stored in a suitable container and preserved with 10 percent formalin or 70 percent ethanol. The samples should be labeled with the location, date, type of sampler used, name of collector, and other pertinent information.

SCI Procedure.

1. Randomize specimens in a jar by swirling.
2. Pour specimens into a lined white enamel pan.
3. Disperse clumps of specimens by pouring preservative or water on the clumps.
4. Determine the number of runs in the sample by comparing two specimens at a time. The current specimen need only be compared with the previous one. If it is similar, it is part of the same run. If not, it is part of a new run (fig. A-1). A 2X magnifying glass or a low-powered binocular microscope is needed for this operation.
5. Determine the total number of specimens in the sample.
6. Calculate DI_1:

$$DI_1 = \frac{\text{number of runs}}{\text{number of specimens}}$$

7. Record the number of different taxa observed. This does not require a specialist in taxonomy. Most bottom fauna organisms are fairly easily divided into recognizable entities by non-biologists. *Identification of the organisms is not necessary.* A 2X magnifying glass or a low powered binocular microscope is needed for this operation.
8. Determine from figure A-2 the number of times (N) the SCI examination must be repeated on the sample to be 95 percent confident that the mean DI_1 is within a desired percentage of the true value for DI_1. In most pollution work involving gross differences, line A of figure A-2 should be used.
9. After determining N from figure A-2, rerandomize the sample and repeat the SCI examination on the same sample N–1 times. Calculate the average DI_1 by the following equation:

$$\overline{DI_1} = \frac{\Sigma DI_1}{N}$$

10. DI_T is a diversity index value. It represents species diversity and, therefore, health of a watercourse. Calculate DI_T by the following equation:

$$DI_T = \overline{DI_1} \times \text{No. of Taxa}$$

11. Repeat the above procedure for each sample collection.

The above procedure should only be used on samples containing fewer than 250 specimens. Healthy streams with a high diversity and a balanced density tend to have DI_T values above 12. Polluted communities tend to have DI_T values of 8 or less. Intermediate values have been found in semipolluted streams.

To determine if different bottom-fauna community structures are significantly different from each other, calculate the 95 percent confidence intervals around each DI_T value. If the intervals do not overlap, then the community structures are significantly different. For example, if sampling station "A" has a DI_T value of 25, station "B" has a DI_T value of 10, and line A of figure A-2 is used, then the 95 percent confidence interval would be 20 percent, or 10 percent on either side of the determined DI_T value. Station "A" has 95 percent confidence interval for the DI_T value from 22.5 to 27.5 (20 percent of 25 = 5). Station "B" has a 95 percent confidence interval for the DI_T value from 9 to 11 (20 percent of 10 = 2). The 95 percent confidence intervals do not overlap, and therefore the bottom fauna communities at the two stations are significantly different.

The SCI examination is a useful tool. It requires no taxonomic expertise. It is easy to perform and produces results quickly. It should not be used to represent or replace other more accurate techniques requiring a person trained in aquatic biology.

Figure A-1

Determination of Runs in Sequential Comparison Index

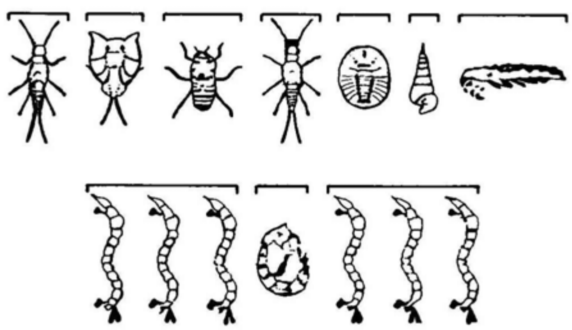
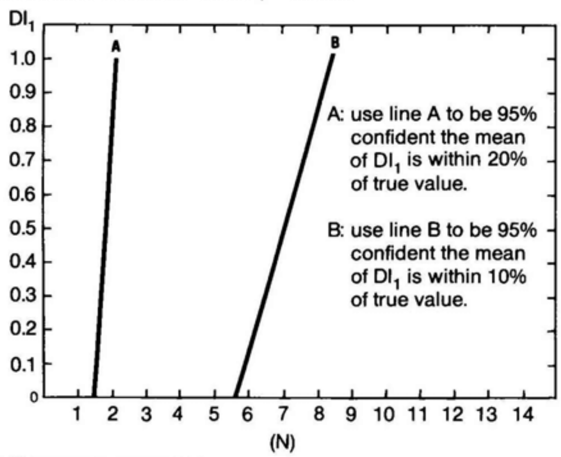


Figure A-2

Confidence Limits for DI_1 Values.



Beck's Biotic Index

Beck's Biotic Index (ref. 3–10) was developed primarily for use in Florida and assumes taxonomic expertise, but it can be used with generic level identification when less sensitivity is acceptable. This system can be used to indicate both the magnitude and probable cause of environmental stress. Beck developed the methodology to categorize stream macro-invertebrates (large animals without backbones, ref. A-1).

Three categories (table A-3, fig. A-4) are defined below:

Class I Organisms (Sensitive or Intolerant)
Organisms that exhibit a rapid response to aquatic environmental changes and are killed, driven out of the area, or as a group are substantially reduced in number when their environment is degraded.

Class II Organisms (Facultative)
Organisms that have the capability to live under varying conditions; e.g., a facultative anaerobe is an organism that although usually and normally lives in the presence of free oxygen, can live in absence of free oxygen. Most survive in areas where organic pollution is producing eutrophication or "enrichment" of the aquatic ecosystem.

Class III Organisms (Tolerant)
Organisms capable of withstanding adverse conditions within the aquatic environment.

According to this approach, which assumes that there are not naturally occurring limiting factors, an undisturbed community will include representatives of the majority of the groups contained in Class I as well as some representatives of Classes II and III. By contrast, a sample which consists mainly of Class II organisms is being "limited" or impacted by either natural factors, such as low flow, homogenous substrate, etc. or is impacted due to human activities. Waters dominated by Class III organisms are probably adversely affected by organic pollution.

The structure of the benthic (bottom) invertebrate community in waterways polluted by organic waste differs quantitatively from invertebrate communities in unpolluted waterways. That is, organic pollution results not just in a reduction in species richness (the total number of benthic groups), but also in a stimulation in density (the total number of organisms collected per sample).

By contrast, waterways impacted with toxic materials, such as pesticides or acid mine drainage, show decreases both in richness and density. Sediment causes a greater reduction in density than in richness. Because of the above differences and because there are often dominant organisms characteristic of sediment pollution, it is possible to differentiate sediment stress from the stresses of toxic materials and organic wastes.

For this type of investigation, a dip net is used to take a "kick" sample, which is sufficient to obtain a representative sample of the organisms present. With this procedure the net is placed upright on the bottom in an area of swift water, and the stream bottom upstream of the net is sufficiently disturbed to dislodge any organisms located there. The dislodged organisms will be carried by the current into the net and captured. Any rocks that can be overturned should be turned and any clinging organisms collected.

Mathematical expression.

$$BI = 2n_{I} + n_{II}$$

where:

BI = Beck's Biotic Index

n_{I} = the number of Class I species identified from the samples

n_{II} = the number of Class II species identified from the samples

Table A-3.—Benthic macroinvertebrates classed according to Beck's Biotic Index Classes (ref. A-2)

Invertebrate Form	Class
Caddisflies: Trichoptera	
Hydropsychidae	1
Hydroptilidae	1
Limnephilidae	1
Leptoceridae	1
Helicopsychiade	1
Psychomyiidae	1
Goeridae	1
Stoneflies: Plecoptera	
Perlidae	1
Perlodidae	1
Mayflies: Ephemeroptera	
Baetidae	1
Heptageniidae	1
Ephemeridae	1
Helligrammites	
Corydalidae	1
Freshwater Naiads (Clams)	
Unionidae	1
Beetles: Coleoptera	
Elmidae (Riffle Beetle)	1
Psephenidae (Water Penny)	1
Damselflies: Odonata	
Coenagrionidae	2
Agrionidae	2
Dragonflies: Odonata	
Aeschnidae	2
Comphidae	2
Libellulidae	2
Crayfish	
Astacidae	2
Flatworms	
Planaridae	2
Crane Flies	
Tipulidae	2
Gill Snails	
Pleuroceridae	2
Horse Flies	
Tabanidae	2
Isopods	
Asellidae (Aquatic Sowbugs)	2
Blackflies	
Simuliidae	2
Air-Breathing Snails	
Physidae	3
Ancylidae (Limpets)	3
Aquatic Earthworms	
Oligochaeta	3
Midges	
Chironomidae	3
Leeches	
Hirundinea	3
Moth flies	
Psychodidae	3

Recommended Level of Taxonomic Identification. This index should be used in conjunction with species level identification to enhance the sensitivity of the index in detecting ecosystem perturbations. The use of generic level identification requires the assignment of a tolerance classification to a genus, corresponding to the most tolerant species within that genus, and leads to decreased index sensitivity. Generic level identification can be utilized when less sensitivity is acceptable or when species identification is not possible. For example, species taxonomy within the *Chironomidae* (midges) can be so difficult as to preclude its use.

Geographic Applicability. This index has not been widely employed outside of the State of Florida.

Computational Devices Required. A simple desk-top calculator is recommended for the calculation of values.

Statistical Evaluation. Statistical evaluation of index values can be inappropriate or present interpretation difficulties. Tests of raw data (Chi square, correlation, t-test, etc.) are recommended.

Use of the index.

1. *Level of sampling required.* Sample size can be limited, depending on the degree of organic pollution encountered. Organically polluted conditions demand more extensive and precise collection and analysis of data to ensure that sensitive animals have not been overlooked.

2. *Recommended form of data reduction.* Absolute estimates of generic and/or specific representation should be entered directly into the computational formula.

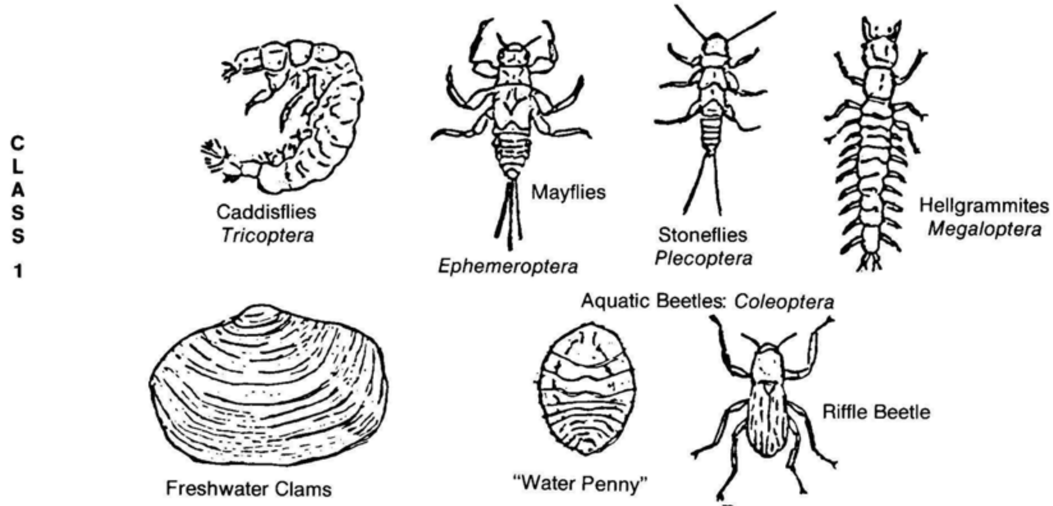
3. *Modes of data display.* Index values can be displayed in either tabular and/or graphical form in a site or locale-specific manner.

4. *Interpretation.* Index values will range from 0 to approximately 40; the lower the index value, the greater the organic stress. See table below. An index value of 10 is the lowest value accepted as indicative of clean water without additional discussion.

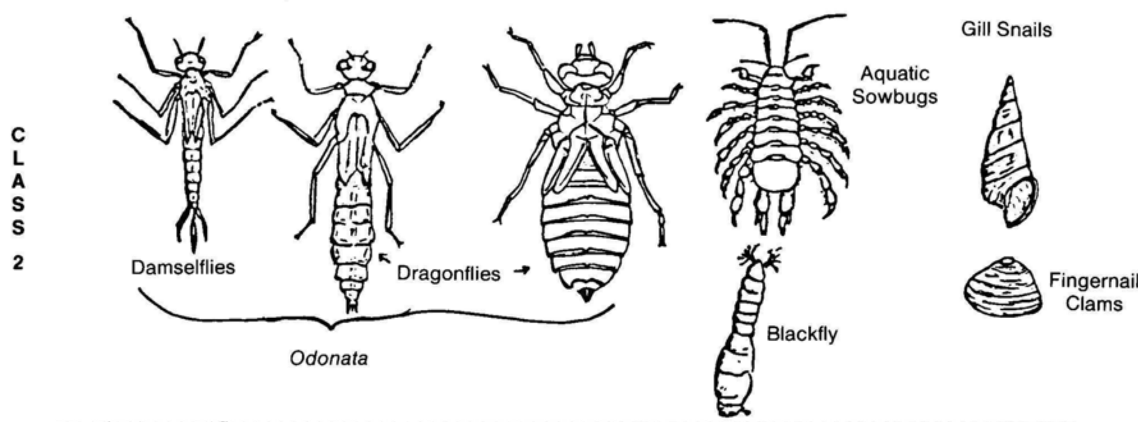
Figure A-4

Macroinvertebrates According to Beck's Biotic Index Classes (Ref. A-2).

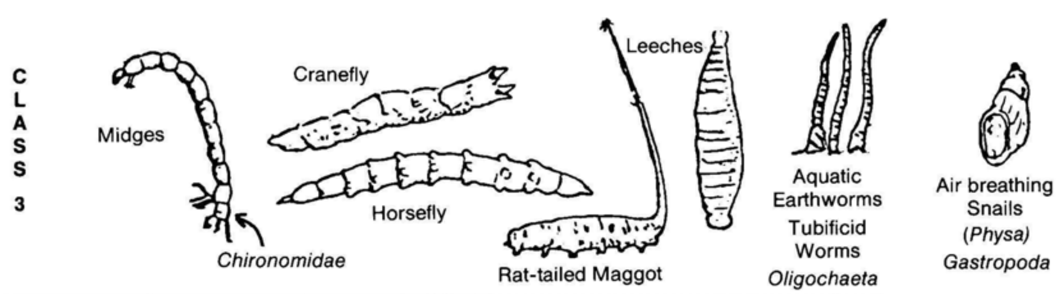
1. Intolerant (sensitive) to pollution:



2. Facultative - Can tolerate some pollution:



3. Tolerant to pollution:



Beck's Biotic Index Values

Index Value	Description
0	Stream grossly polluted
1 to 5	Stream moderately polluted
6 to 9	Stream clean, but with a monotonous habitat and instream velocity
10 or greater	Stream clean

Floating Body Technique

The Floating Body Technique measures water flow velocity, which is calculated by measuring the time taken for a marker to travel a known distance downstream.

Procedure. A stretch of a watercourse should be selected which is approximately straight. The compass direction of this stretch should be measured. Using the compass direction, a 90 degree angle is laid out so it crosses the stream. This is most conveniently done by locating a landmark on the distant side of the stream, and moving up- or downstream to locate (and mark) the point at which a 90 degree angle exists. A distance should then be measured downstream to the other end of the straight stretch, and a similar 90 degree angle laid out. The marker or dye is tossed into the stream above the initial point and then timed to see how long it takes to get from one point to the other. If dye is used, the time is measured until the front part of the dye stain arrives. Velocity is calculated as distance divided by time.

When the velocity measured is the peak velocity of the stream (usually at the surface in the center), it is possible to calculate an approximate average for velocity for the stream, assuming typical cross section. A common average is 85 percent of the maximum current velocity.

Accuracy. This technique can be moderately accurate. The major sources of error are caused by the marker floating out of the desired path. If the maximum current velocity is desired, the marker may tend to end up in eddies along the way, rather than staying in the maximum velocity portion of the stream. This source of error can be reduced by making repeated measurements or by using dye as the marker. Calculations of average stream velocity from a measured maximum velocity are in error if the correction factor is inappropriate. Deviations from the 85 percent factor mentioned above are common.

Application Notes. This technique is inexpensive. A crew size of one is suitable for slow moving streams, but a crew size of two is necessary to signal when the marker has passed the ending point if the stream moves too fast for one crew member to move from the starting point to the ending point. It is most appropriate where streams are relatively large and have a smooth slope.